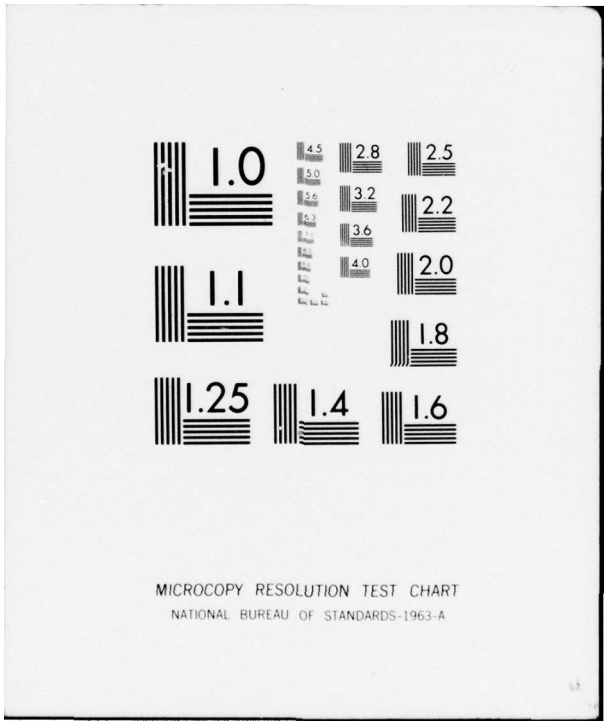
AD-A040 367 DESERET TEST CENTER FORT DOUGLAS UTAH F/G 6/3
EFFECTS OF SEVERAL WETTING AGENTS ON THE VIABILITY OF SOME ARTH--ETC(U)
APR 70 G F ORR, D S THORNE, W C TIPPETS

UNCLASSIFIED DTC-TB-50 NL

| OF |
AD A040367

END

DATE FILMED
7-77


1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6
6.3
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

AD
RDTE PROJECT NO. 1X665704D63408
USATECOM PROJECT NO. 5-CO-413-000-015
DTC PROJECT NO. DTC-TB-50

TECHNICAL NOTE

EFFECTS OF SEVERAL WETTING AGENTS ON THE VIABILITY OF SOME ARTHROALEURIOSPOROUS FUNGI

by

G. F. Orr
D. S. Thorne
W. C. Tippets

April 1970

24 p.

DESERET TEST CENTER
Fort Douglas, Utah 84113

ABSTRACT

Asexual spores of Auxarthron reticulatum were suspended in controls of distilled water and physiological saline and in a 0.5% concentration of several wetting agents including Tergitol 4, Nalquot, sodium lauryl sulfate, dioctyl sodium sulfosuccinate, triethanolamine oleate and Roccal. Suspensions were mixed by sonic and hand agitation. Viability of these spores was completely inhibited by Roccal and markedly reduced by Nalquot and dioctyl sodium sulfosuccinate. Spores suspended in sodium lauryl sulfate and triethanolamine oleate exhibited viabilities greater than those of spores suspended in distilled water or physiological saline. The viability (stimulation) of spores suspended in Tergitol 4 was increased approximately tenfold over that of the controls.

Asexual spores of A. reticulatum, Malbranchea sp. and Coccidioides immitis were suspended in various concentrations of Tergitol 4 (0.5% to 15%). A. reticulatum exhibited little reduction of viability at 0.5%, 1%, 1.5% and 5%. Some possible stimulation was observed at 2%; viability was reduced approximately tenfold at 10% and 15%. Viability of spores of Malbranchea sp. was shown to be higher in all concentrations of Tergitol 4 than in distilled water and physiological saline, but the greater viability was exhibited at the 12% and 15% concentrations. C. immitis exhibited a reduction of viability of approximately 80% at the 1% and 1.5% concentrations and by nearly one hundredfold at the 5% concentration of Tergitol 4. Complete inhibition was observed at concentrations greater than 5%.

The use of appropriate concentrations of Tergitol 4 as a stimulator in growth medium or as a collecting fluid additive for certain arthroaleuriosporous fungi is suggested.

TABLE OF CONTENTS

I. INTRODUCTION

In preparing suspensions of fungus spores in water and various other fluids, inadequate dispersion is often observed. In many cases, dry harvested spores tend to rise to the liquid surface shortly after the initial dispersion causing difficulties in dispensing, plating and counting of some colonies. Common fluids in the laboratory that aid in spore dispersion (ethanol, acetone) are frequently deleterious to the spores even at low concentrations. Certain military specifications advise the use of specific wetting agents such as dioctyl sodium sulfosuccinate (0.005%) as a dispersing aid (1)*.

Among the fungi exhibiting flocculation and poor dispersing characteristics in untreated fluids are the arthroaleuriospore-formers; Gymnoascaceae, species of Malbranchea and Coccidioides immitis. "Arthroaleuriospore" is a term coined by Orr et al. (2) for those spores that resemble arthorspores but which are formed and released by a process similar to that of aleuriospores.

This study was undertaken to provide information regarding effects of certain wetting agents on spore dispersion that may improve certain phases of quantitative laboratory work.

* Literature cited.

II. MATERIALS AND METHODS

Fungi utilized in this study were grown on Sabouraud's Glucose Agar fortified - SAB-PSA[2] (3) at 30°C for 45-60 days or until dry, harvested by a vacuum-type harvester and stored in sterile 4-oz screw-capped jars at 4°C until required.

TREATMENT #1:

a. Dry harvested arthroaleuriospores (0.1 gm) of Auxarthron reticulatum (Strain 0-1020, isolated from wood, southern California) was added to 9.9 ml of each of the following solutions prepared in duplicate; Control #1 - distilled water, Control #2 - physiological saline (0.85% NaCl) and 0.5% solutions of Tergitol 4 Anionic (Union Carbide Co.), Nalquot (source unknown), diocty1 sodium sulfosuccinate-DSS (Aerosol - OT, Van Waters & Rogers), Sodium lauryl sulfate-SLS (Fisher Scientific Co.). Triethanolamine oleate - TEA (Chemical Division, Deseret Test Center) and Roccal (Winthrop Laboratories).

b. One set of tubes of the above spore suspensions was shaken by tapping the tube against the side of the hand for 10-15 minutes or until visible clumps were disintegrated.

c. The other set of tubes of the spore suspensions was placed in a water bath and agitated by a sonic vibrator (Branson Instruments Inc.) for 10-15 minutes.

d. Each suspension was examined macroscopically and microscopically and arthroaleuriospores were counted using a hemacytometer.

e. All suspensions were plated in duplicate per standard laboratory methods (DPC SOP #8) on SAB-PSA[2] (3), incubated at 30°C for 4-6 days, and colonies of A. reticulatum were counted and recorded.

TREATMENT #2:

a. Dry harvested arthroaleuriospores (0.1 gm) of A. reticulatum (Stain 0-1020), Malbranchea sp. (Strain 0-837, isolated from coyote dung, southern California) and Coccidioides immitis (Strain SD-1, isolated from soil, San Diego County, California) were suspended in sterile distilled water and various concentrations (0.5 - 15%) of Tergitol 4. A. reticulatum and Malbranchea sp. were also suspended in physiological saline. All suspensions were mixed for 5-10 minutes on a Vortex-type mixer.

b. Each suspension was diluted serially to 10^{-10}, plated in duplicate per standard laboratory methods on SAB-PSA[2]. Plates were incubated at 30°C for 4-8 days; colonies of the specific fungi were counted and recorded.

Four or more trials were done for each of the three fungi.

III. RESULTS AND DISCUSSION

Flocculation and poor dispersal of arthroaleuriospores in fluids may result because of air trappage in the remnants of the connective cell between them or at the base of a terminal spore (Figs. 1, 2, 3, 4). Air becoming trapped in the skirt-like projection at either end of the arthroaleuriospore would thus force it to the surface of the fluid after initial dispersion efforts. The spore wall is apparently impervious to liquid water, but not to gaseous water since these spores tend to swell somewhat prior to germination in a humid atmosphere. Possibly, these "skirted" ends act aerodynamically in dispersion under natural conditions permitting the spore to travel end-over-end through the air.

Macroscopically, Tergitol 4, Nalquot, SLS, TEA and Roccal provided about equal dispersion by hand agitation; DSS was somewhat poorer. Spore material in the control fluids (distilled water and physiological saline) tended to surface rapidly with but little of the material remaining in suspension. Spores in the wetting agents tested demonstrated improved dispersion after sonic agitation, although spores in distilled water and physiological saline exhibited little, if any, improvement in dispersion.

Spore suspensions were also examined microscopically after sonic agitation. Tergitol 4, Nalquot, and SLS appeared to be about equal in dispersion ability. Suspensions in Roccal and DSS were somewhat cloudy and created some difficulty in counting. Spores suspended in TEA were less well dispersed and this material was unsatisfactory for counting because of the presence of numerous large oil drops. Hand agitation generally provided considerably less dispersion of the spores, except in SLS.

That the use of wetting agents aided dispersion of the spores is evident in the physical counts (Table I), all of which were approximately tenfold higher than either of the controls. Sonic agitation counts were also generally higher than counts from suspensions agitated by hand except those in physiological saline which were only slightly lower. In distilled water, TEA and SLS, physical counts of spores were nearly the same by either method of agitation. In Tergitol 4, Nalquot and Roccal, physical counts of spores by hemacytometer were from two to approximately four times higher by sonic agitation than those counts obtained from hand agitation.

Viability of the arthroaleuriospores was markedly reduced by Nalquot and DSS (Table I) and was completely inhibited by Roccal. Apparently,

concentrations of DSS higher than that specified (0.005%) by the military specifications (1) for wetting fungus spores in suspension is deleterious. The efficiency of Roccal as a decontaminating agent is supported in this work.

The greatest viability count of all wetting agents tested was exhibited by spores in Tergitol 4 which was approximately three times greater than that shown by SLS and TEA; and nearly ten times greater than that exhibited by spores in distilled water and physiological saline. However, spores suspended in distilled water and physiological saline exhibited greater percent viability (46.2% and 39.6% by sonic agitation and 64.0% and 56.4% by hand agitation respectively) with respect to their physical counts (Table II). The latter fluids, however, provided less satisfactory dispersing properties to the spores than did the wetting agents. Reduced viability, in part, may have resulted from the spore harvesting technique and storage.

Viability counts of spores under hand agitation in distilled water and physiological saline were greater than those under sonic agitation. Possible harmful effects might occur if sonic agitation was prolonged. Little significant difference was observed, however, between viability counts of spores agitated by either method in SLC or DSS. That hand agitation would be less deleterious to spores would be compensated for by the greatly improved dispersion in wetting agents, especially Tergitol 4.

The three arthroaleuriosporous fungi utilized in Treatment #2 presented some difficulties in counting due to size, coloration and shape (1.2 - 4.8 x 2.8 - 8.2 microns, hyaline to very pale yellow when viewed singly, cylindrical to more or less barrel-shaped), especially when observed in groups. Physical counts were found to be quite variable and often less than the viability counts. Although dispersion was good, especially in Tergitol 4, visual counting was considered inadequate. Vortex-type agitation appeared to be quite satisfactory.

Spores of A. reticulatum demonstrated lower viability in physiological saline than in distilled water by approximately three times in Treatment #2 (Table III) differing from the same material in Treatment #1. The reason for this difference is unknown. Figures given (Table III) are averages of four or more trials. Considerable variation of viability counts was demonstrated between 0.5% and 5.0%, concentrations of Tergitol 4, with some slight stimulation at the 2.0% level. Decreases in viability were observed only at the 10% and 15% levels (approximately ten-fold). It appears that Tergitol 4 has but little effect on the viability of spores of A. reticulatum at most con-

centrations used. Perhaps this wetting agent could be utilized in conjunction with a growth medium as a stimulator.

Malbranchea sp. exhibited a different aspect in that the greatest viability of spores was demonstrated at the highest concentrations of Tergitol 4, 12% and 15%; and less stimulation was observed at the lowest concentrations, 0.5% and 1.0% (Table III). Viabilities of spores were equal in distilled water and physiological saline, but were lower in these two fluids than in all concentrations of Tergitol 4. This finding suggests that Tergitol 4 is stimulatory to the spores of this species of Malbranchea. However, viability of the spores of this organism was ten to a thousand-fold less under all conditions than that demonstrated by the spores of A. reticulatum. Possibly, the spore harvesting procedure was more severe for this Malbranchea sp. than for A. reticulatum.

Not only was viability even more reduced for spores of C. immitis than for the other two fungus species, but Tergitol 4 was apparently inhibitory at concentrations above 5%; complete inhibition occurring at 10% and 15%. (Table III). Viability of the spores at the 0.5%, 1.5% and 5.0% levels of Tergitol 4 was considerably less (eight to fifteen times) than that of the control in distilled water. Not only the spore harvesting technique, but the stringent safety procedures required may have caused a reduction in the viability of the spores of C. immitis.

Physiological saline was not utilized in Treatment #2 for C. immitis because of the work done by Friedman et al. (4) who suspended arthroaleuriospores of C. immitis in brine at 4°C and at room temperature. No loss in viability was demonstrated for at least thirty days. Other studies regarding little viability loss or only slow decreases in viability of spores of C. immitis over prolonged periods include those of Elconin et al. (5) and Orr et al. (6).

Since the asexual spores (arthoaleuriospores) of species of Malbranchea, Auxarthron reticulatum and other species of the Gymnoascaceae are similar to those produced by Coccidioides immitis (fungal agent causing "Valley Fever") and because of restrictions recently placed on the testing of pathogens, the fungus specimens named above might be considered as potential simulant candidates for C. immitis. Simulants of this sort could provide necessary information for the determination of cloud behavior under various sets of conditions which could then be applied to probable behavior of C. immitis under similar conditions. Should such species as those studied in this work become considered as candidate simulants, addition of appropriate concentra-

tions of Tergitol 4 might provide an improved growth medium, used as a stimulatory additive or as a portion of a collecting fluid. Although foaming would probably occur in impinger samplers containing Tergitol 4, appropriate antifoam agents could be utilized. Improvements in dispersion of collected spores by this wetting agent might provide more reliable quantitative data.

IV. SUMMARY

Asexual spores of Auxarthron reticulatum were suspended in distilled water, physiological saline and a 0.5% concentration of several wetting agents and mixed by sonic and hand agitation. Wetting agents included Tergitol 4, Nalquot, sodium lauryl sulfate, dioctyl sodium sulfosuccinate, triethanolamine oleate and Roccal. Viability of these spores was completely inhibited by Roccal and markedly reduced by Nalquot and dioctyl sodium sulfosuccinate. Spores suspended in sodium lauryl sulfate and triethanolamine oleate exhibited viabilities greater than those of spores suspended in distilled water and physiological saline. Increased viability (stimulation) was demonstrated by spores suspended in Tergitol 4 (approximately seven times greater).

Asexual spores of Auxarthron reticulatum, Malbranchea sp. and Coccidioides immitis were suspended in various concentrations of Tergitol 4 (0.5% - 15.0%). A. reticulatum exhibited little reduction of viability at 0.5%, 1.0%, 1.5% and 5.0%. Some possible stimulation was observed at the 2.0% level; viability was reduced approximately tenfold at 12% and 15%. Viability of spores of Malbranchea sp. was found to be higher in all concentrations of Tergitol 4 than in distilled water and physiological saline, but the greatest stimulatory effect was exhibited at the 12% and 15% levels. Spores of C. immitis exhibited a viability reduction by approximately 80% at the 1.0% and 1.5% levels and by nearly one hundredfold at the 5.0% level. Complete inhibition was observed at all concentrations above 5%.

The use of appropriate levels of Tergitol 4 as a stimulatory additive in growth media or collection fluid is suggested.

ACKNOWLEDGEMENT

The writers wish to express their thanks to Dr. H. E. Thompson, Chemical Division, Deseret Test Center, Dugway Proving Ground, for preparation of the triethanolamine oleate. Special thanks are due to Mr. Dennis Morgan, Drafting Branch, Deseret Test Center, Dugway Proving Ground, for preparation of the diagrammatic sketches.

Coccidioides immitis spore viability was reduced

LITERATURE CITED

1. General specification for fungus resistance tests, aeronautical and associated materiels. MIL-F-8261A (USAF). 1955.

2. Orr, G. F., H. H. Kuehn and O. A. Plunkett. 1963. The genus Myxotrichum Kunze. Can. J. Bot. 41: 1457-1480.

3. Orr, G. F. 1968. Some fungi isolated with Coccidioides immitis from soils of endemic soils in California. Bull. Torrey Bot. Club 95: 424-431.

4. Friedman, L. C., C. E. Smith and R. H. Berman. 1962. Studies on the survival characteristics of the parasitic phase of Coccidioides immitis with comments on contagion. Am. Rev. Resp. Dis. 85: 224-231.

5. Elconin, A. F., R. O. Egeberg and M. C. Egeberg. 1964. Significance of soil salinity on the ecology of Coccidioides immitis. J. Bact. 87: 500-503.

6. Orr, G. F., D. S. Thorne and W. C. Tippets, 1968. Persistency of Coccidioides immitis in Dugway soils under laboratory conditions. DPG Technical Report #T68-108. 20 pp.

TABLE I

DISPERSION AND TOXICITY OF A 0.5% CONCENTRATION OF SEVERAL WETTING AGENTS ON ARTHROALEURIOSPORES OF AUXARTHRON RETICULATUM

Wetting Agent	Physical Counts ($\times 10^9$ particles/gm)		Viability Counts ($\times 10^9$ particles/gm)	
	Sonic Agitation	Hand Agitation	Sonic Agitation	Hand Agitation
DHOH (Control #1)	6.5	5.0	3.0	3.2
PS (Control #2)	5.3	7.8	2.1	4.4
TER	62.0	39.0*	23.0	95.0*
NQ	67.0	37.0	0.0068	0.021
DSS	68.0	18.0	0.0022	0.0065
SLS	54.0	50.0	9.3	9.2
TEA	41.0	37.0	9.6	9.2
ROC	61.0	35.0	0.0	0.0

LEGEND:

* Possible miscount of particles prior to viability check. Compare with both counts from sonic agitation.

DHOH Distilled water

PS Physiological saline

TER Tergitol

NQ Nalquot

DSS Dioctyl sodium sulfosuccinate

SLS Sodium lauryl sulfate

TEA Triethanolamine oleate

ROC Roccal

TABLE II

COMPARISON OF VIABILITY (PERCENT) TO PHYSICAL COUNTS OF A 0.5% CONCENTRATION OF SEVERAL WETTING AGENTS ON ARTHROALEURIOSPORES OF AUXARTHRON RETICULATUM

WETTING AGENT	SONIC AGITATION	HAND AGITATION
HOH (Control #1)	46.2	64.0
PS (Control #2)	39.6	56.4
TER	37.1	306.0*
NQ	0.01	0.06
DSS	0.03	0.04
SLS	17.2	18.4
TEA	23.4	24.9
ROC	0.0	0.0

* Probable miscount of particles physically.

LEGEND: See Table I

TABLE III

EFFECTS OF VARIOUS CONCENTRATIONS OF TERGITOL ON VIABILITY OF THREE ALEURIOSPOROUS FUNGI

Fungus Strain	Tergitol Concentrations (%)									
	DHOH (Control #1)	PS (Control #2)	0.5	1.0	1.5	2.0	5.0	10.0	12.0	15.0
0-1020	6600.0*	2100.0	6000.0	3700.0	2400.0	7100.0	1900.0	270.0	-	170.0
0-837	0.72	0.72	1.70	1.60	-	2.50	3.40	3.40	16.0	31.0
SD-1	12.0	-	2.0	-	2.20	-	0.80	0.0	-	0.0

LEGEND:

* Viability counts = x 10^9 particles/gm

DHOH Distilled water

PS Physiological saline

0-1020 Auxarthron reticulatum

0-837 Malbranchea sp.

SD-1 Coccidioides immitis

\- Not done

EXPLANATION OF FIGURES

Fig. 1 Photomicrograph of arthroaleuriospores of Coccidioides immitis stained by methylene blue. Note the more or less barrel-like shapes and the empty connective cells. A loose arthroaleuriospore is visible at the lower left of the main chain. The "skirt-like" remainder of the connective cell is visible on two of the arthroaleuriospores. Original magnification - 1000x.

Fig. 2 Diagramatic sketch of the same arthroaleuriospore chain.
- a. A terminal arthroaleuriospore.
- b. Empty connective cell.
- c. Intercalary arthroaleuriospore.
- d. Thickened surface interior of primary spore wall.

Fig. 3 Diagramatic sketch of a terminal arthroaleuriospore. This spore cannot be distinguished from a normal aleuriospore.
- a. Primary spore wall, exterior.
- b. Thickened interior surface of the primary spore wall.
- c. Primary spore wall of connective cell remaining as a "skirt-like" protuberance.
- d. Open end of "skirt" that can trap air when in suspension in fluids or possibly act aerodynamically under natural conditions.

Fig. 4 Diagramatic sketch of a typical arthroaleuriospore. Note the "skirt" at either end.
- a, d. Primary wall of empty intercalary connective cells.
- b, e. Open end of "skirts" that permit trapping of air in fluids and possibly behave aerodynamically under natural conditions.
- c. Reproductive or germinative portion of arthroaleuriospore.

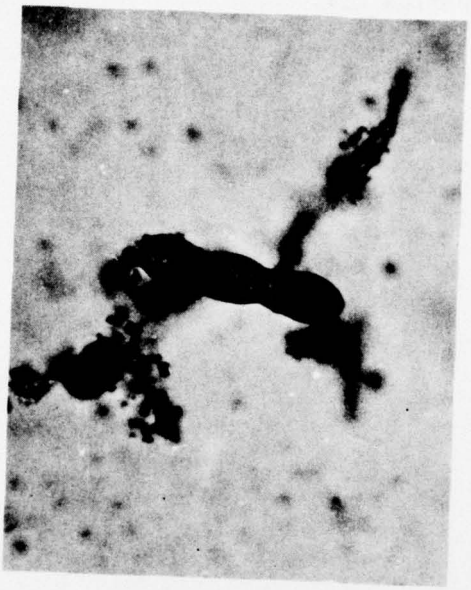


Fig. 1

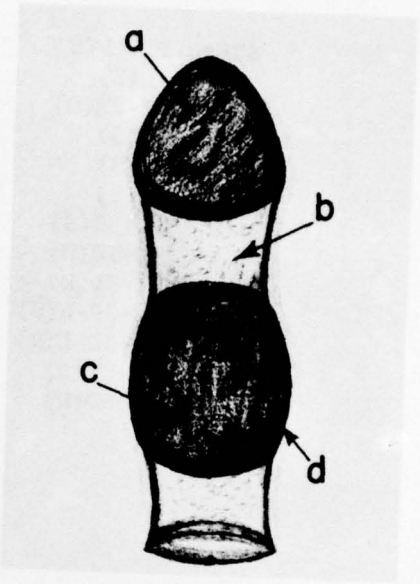


Fig. 2

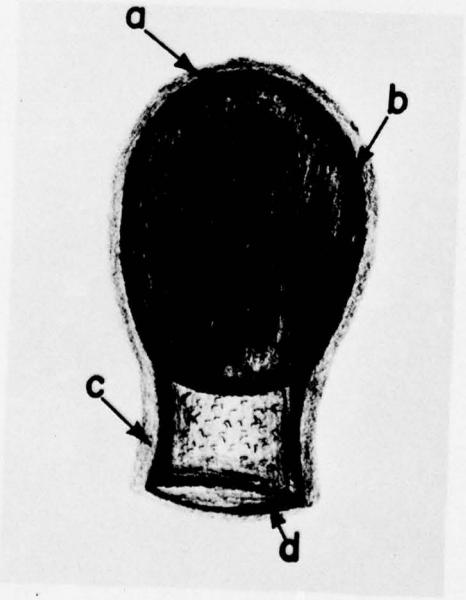


Fig. 3

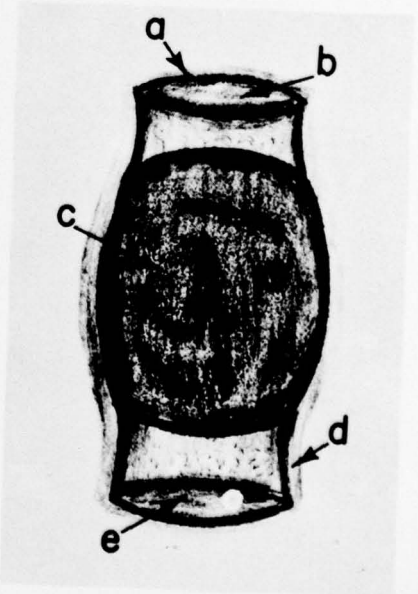


Fig. 4

DISTRIBUTION LIST

STEPD-PT-AD(S)	1
SC(S)	1
PT-RM(D)	1
TT(S)	1
TO(S)	1
PA(S)	1
PA-BS(S)	1
TO-TD(D)	1
TT-CD(D)	1
TT-JP-L(S)	5
TT-JP-L(D)	1
PA-MT(D)	1
TT-BD(D)	23

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Biological Division Technology & Technical Support Dir Deseret Test Center, Dugway, Utah	Unclassified 2b. GROUP

3. REPORT TITLE

EFFECTS OF SEVERAL WETTING AGENTS ON THE VIABILITY OF SOME ARTHROALEURIOSPOROUS FUNGI

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Technical Note

5. AUTHOR(S) (First name, middle initial, last name)
G. F. Orr
D. S. Thorne
W. C. Tippets

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
April 1970	20	6

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. RDT&E # 1X665704D63408	DTC TB - 50
c. TRMS # 5-CO-413-000-015	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	

10. DISTRIBUTION STATEMENT
This document may be further distributed by any holder only with specific prior approval of Commanding Officer, Deseret Test Center, ATTN: STEDP-SA-I(S), Fort Douglas, Utah 84113.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY

13. ABSTRACT

Asexual spores of Auxarthron reticulatum were suspended in controls of distilled water and physiological saline and in a 0.5% concentration of several wetting agents including Tergitol 4, Nalquot, sodium lauryl sulfate, dioctyl sodium sulfosuccinate, triethanolamine oleate and Roccal. Suspensions were mixed by sonic and hand agitation. Viability of these spores was completely inhibited by Roccal and markedly reduced by Nalquot and dioctyl sodium sulfosuccinate. Spores suspended in sodium lauryl sulfate and triethanolamine oleate exhibited viabilities greater than those of spores suspended in distilled water or physiological saline. The viability (stimulation) of spores suspended in Tergitol 4 was increased approximately tenfold over that of the controls.

Asexual spores of A. reticulatum, Malbranchea sp. and Coccidioides immitis were suspended in various concentrations of Tergitol 4 (0.5% to 15%). A. reticulatum exhibited little reduction of viability at 0.5%, 1%, 1.5% and 5%. Some possible stimulation was observed at 2%; viability was reduced approximately tenfold at 10% and 15%. Viability of spores of Malbranchea sp. was shown to be higher in all concentrations of Tergitol 4 than in distilled water and physiological saline, but the greater viability was exhibited at the 12% and 15% concentrations. C. immitis exhibited a reduction of viability of approximately 80% at the 1% and 1.5% concentrations and by nearly one hundredfold at the 5% concentration of Tergitol 4. Complete inhibition was observed at concentrations greater than 5%.

The use of appropriate concentrations of Tergitol 4 as a stimulator in growth medium or as a collecting fluid additive for certain arthroaleuriosporous fungi is suggested.

DD FORM 1 NOV 68 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Arthroaleuriosporous fungi						
Wetting agents						
Stimulation						
Inhibition						
C. immitis						
Simulants						